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Invasion or Progression

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Washington, DC 20306

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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) To assess interactions between epithelial (EP) and myoepithelial (ME) cells in association with breast tumor progression and invasion, consecutive sections were immunostained with antibodies to estrogen receptor (ER), smooth muscle actin (SMA), and other biomolecules, and then microdissected for LOH and microsatellite instability (MI) assessments. Focal losses of ER expression in EP cells and disruptions of subjacent ME layers are correlated events in ER (+) tumors, whereas focal alterations of p27 expression in EP cells and disruptions of subjacent ME layers are correlated events in ER (-) tumors, suggesting that progression or invasion of these tumors may be regulated by different mechanisms. Cells in ducts with disrupted ME cell layers showed a substantially higher proliferation rate, and a vast majority of ER (-) cells overlying disrupted ME cell layers showed a marked higher frequency and different pattern of LOH and MI, compared to adjacent ER (+) cells within the same duct. These findings are in an agreement with our hypothesis that ER (-) cells overlying disrupted ME cell layer represent a more aggressive clone, and that simultaneous assessments of the immunohistochemical and genetic profiles of EP and ME cells could be a more sensitive approach for early detection of breast tumor progression or invasion.				
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## **Introduction**

To assess interactions between epithelial (EP) and myoepithelial (ME) cells in association with breast tumor progression and invasion, a double immunostaining technique with antibodies to smooth muscle actin (SMA) and estrogen receptor (ER) was used to elucidate both the ME and EP cells in mammary tissues harboring ductal carcinoma in situ. Single or clusters of EP cells with a marked diminution or a total loss of ER expression were found immediately overlying focally disrupted ME cell layers, in contrast to the dominant population of ER (+) cells within the same duct that showed no associated ME cell layer disruptions (1). This study attempted to confirm our previous findings on a larger number of cases, and to compare the immunohistochemical and molecular biological profiles of the ER (-) cells overlying disrupted ME cell layers with those of adjacent ER (+) cells and surrounding stromal (ST) cells. Since ME cell layers are physical barriers protecting the microenvironment and integrity of EP cells, and the disruption of ME cell layers is an absolute pre-requisite for breast tumor invasion, the outcomes of this project could have significant values in early detection of breast tumor progression and/or invasion.

## **Body**

### **Statement of work**

A total of 7 tasks were listed in the Statement of Work of the original proposal:

- Task 1. To repeat our previous studies and to identify epithelial (EP) cells overlying disrupted myoepithelial (ME) cell layers (months 1-6).
- Select 500 female cases of ductal carcinoma in situ (DCIS) from our files with detailed information regarding age, race, and follow-up data
  - Retrieve paraffin and frozen tissue blocks, and make 6-8 serial sections for each case
  - Stain the first and the last sections of each case with H & E for morphological assessment
  - Immunostain 3-4 sections from each case with antibodies to estrogen receptor (ER) and smooth muscle actin (SMA)
  - Observe stained sections to identify cells overlying disrupted ME cell layers
  - Select the cases with cells overlying disrupted ME cell layers
- Task 2. To compare the biological behavior of cells overlying a disrupted ME cell layer with that of adjacent cells within the same duct (months 6-9)
- Make 40-50 serial sections for each of the selected cases
  - Immunostain sections with different bio-markers that have been found associated with more aggressive biological behavior
- Task 3. To microdissect phenotypically different EP cells and the surrounding ME and stromal (ST) cells for molecular biological analyses (months 9-12)
- Task 4. To compare the frequency and pattern of loss of heterozygosity (LOH) and clonality among EP, ME, and ST cells (months 12-20)
- Task 5. To assess the gene expression pattern in cells from frozen section sections with cDNA expression array technique, and to generate probes based on sequences exclusively or mainly expressed in cells overlying disrupted ME cell layers (months 20-24)

Task 6. To apply the probes to both paraffin and frozen sections, to identify the gene expressing cells and their morphologic features (months 24-32)

Task 7. To correlate the laboratory findings with that of clinical following-up data (months 32-36).

### **Experimental procedures:**

Consecutive sections were made from formalin-fixed, paraffin-embedded breast tissues from patients with various grades of ductal carcinoma in situ (DCIS), and double immunostained for ER and SMA. Cross sections of all ducts lined by  $\geq 40$  EP cells were examined for a focal ME cell layer disruption, defined as an absence of ME cells, resulting in a gap equal to or greater than the combined size of 3 EP or ME cells. A focal loss of ER expression was defined as marked diminution or a total loss of ER staining in cells immediately overlying a disrupted ME cell layer, in contrast to strong ER expression in adjacent cells within the same duct.

Consecutive sections were also immunohistochemically stained with different antibodies, as detailed in References 5-7, to assess the biologic behavior of cells associated with disrupted ME cell layers. In addition, after immunostaining for ER and SMA, cells overlying disrupted ME cell layers, adjacent ER (+) cells within the same duct, adjacent stromal (ST) cells, and other controls were microdissected for DNA extraction and assessments for loss of heterozygosity (LOH) and microsatellite instability (MI), using PCR amplification with different DNA markers at different chromosomes. The frequency and pattern of LOH and MI among samples were compared.

All above experimental procedures had been carried out according to the methods described in the proposal without any major modifications. Also, all the laboratory efforts have been strictly adhered to address the issues listed in "Objectives" and "Statement of Work" of the original proposal.

### **Key research accomplishments**

All the laboratory procedures for Tasks 1, 2, and 3 had been completed; for Task 4 had been partially completed before July 22, 2002. Also, a majority of the completed experimental materials had been analyzed before July 22, 2002.

### **Reportable outcomes**

Two abstracts that summarized the immunohistochemical findings and the principal molecular techniques for Tasks 1 to 4 were accepted for slide and poster presentations at two major international conferences, and published in Med-Line listed journals (2-3).

Six abstracts that addressed the issues listed in Tasks 1 to 4 of "Statement of Work" have been accepted for poster presentation at Era of Hope, The Department of Defense Breast Cancer Research Program Meeting, to be held in Orlando, Florida, September 25-28, 2002 (4-9).

One additional abstract addressing the issues listed in Tasks 1 to 4 of "Statement of Work" has been sent to 25<sup>th</sup> Annual San Antonio Breast Cancer Symposium to be held in San Antonio, TX this December (10).

The corresponding manuscript for each of the 9 abstracts is under preparation, and will be sent for publication before the end of the year 2002.

In addition, the author has contributed to 12 published or submitted articles during 2001-2002 (11-22)

## **Conclusions**

1. Of 220 ER (+) cases with a total of 5,698 duct cross sections examined so far, 94 (42.7%) contained disrupted ME cell layers with a total of 405 focal disruptions. Of these disruptions, 350 (86.4%) were subjacent to cells with focal losses of ER expression, while only 55 (13.6%) were associated with cells showing a strong ER expression (3-4). These findings are consistent with those of our previous studies (1), suggesting that focal losses of ER expression in EP cells and disruptions of subjacent ME cell layers are correlated events in ER (+) tumors.
2. Of 100 cases with various grades of ER (-) tumors evaluated so far, focal disruptions of ME cell layers were found in about a half of the cases. These focal ME cell layer disruptions, however, appeared to correlate with either a focal loss or elevation of p27 expression (5), suggesting that the progression and/or invasion of ER (-) tumors might differ from those of ER (+) tumors.
3. Several tumor suppressor gene products have been found co-expressed in ME cell layers, and a diminution or absence of these proteins correlated with an increased frequency of ME cell layer disruptions (6-7). A substantially higher cell proliferation rate was seen in ducts with disrupted ME cell layers than ducts with intact ME cell layers (6-7), suggesting that EP cells overlying disrupted ME cell layers may have a more aggressive biologic behavior.
4. As previous studies have shown that it is difficult or impossible to utilize immunostained tissues pre-treated with antigen unmasking methods for molecular analyses, an innovative antigen retrieval protocol that satisfies both immunohistochemical and subsequent molecular assessments has been developed in our laboratory (8). This protocol allows us to microdissect double immunostained cells for LOH and MI assessments, to assess the possible correlation between immunohistochemical and genetic alterations.
5. A vast majority of the ER (-) cells overlying disrupted ME cell layers showed a substantially higher frequency and different pattern of LOH and MI, compared to adjacent ER (+) counterparts within the same duct (9). In a small proportion of cases, however, ER (-) cells showed a substantially lower frequency of LOH and MI than adjacent ER (+) cells, or even displayed no distinct genetic changes (9). These findings are largely in support of our hypothesis that ER (-) cells overlying disrupted ME cell layers represent a more aggressive clone, while also suggest that a few of these cells might belong to a population involving in a normal replenishment or expansion of ducts.

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**Immunohistochemical and genetic alterations in mammary epithelial cells immediately overlying focally disrupted myoepithelial cell layers**

Man YG, Shekitka KM, Brattbauer GL, Tavassoli FA. Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC 20306-6000.

**Background:** Our previous studies, using a double immunostaining technique with antibodies to smooth muscle actin and estrogen receptor (ER), revealed that focal losses of ER expression in epithelial (EP) cells and disruptions of the subjacent myoepithelial (ME) cell layers were correlated events in ER (+) non-invasive breast lesions. This study attempted to confirm this finding and to assess the genetic profiles of cells overlying disrupted ME cell layers.

**Materials and Methods:** Tissue sections were made from 220 patients with various types of breast lesions and were double immunostained with the same protocol. Cross sections of ducts lined by  $\geq 40$  EP cells were examined for focal ME cell layer disruptions, defined as an absence of ME cells, resulting in a gap equal to or greater than the combined size of 3 EP or ME cells. EP cells immediately overlying disrupted ME cell layers and adjacent EP cells within the same duct were microdissected for loss of heterozygosity (LOH) and microsatellite instability (MI) assessment.

**Results:** Of 5,698 duct cross sections examined, 405 focal disruptions were detected. Of which, 350 (86.4%) were subjacent to cells with a loss of ER expression, while 55 (13.6%) were subjacent to cells with a high level of ER expression. A vast majority of ER (-) cells immediately overlying disrupted ME cell layers displayed a substantially higher frequency or different pattern of LOH and MI, compared to adjacent ER (+) cells within the same duct, while in a small proportion of cases, ER (-) cells showed a marked lower or even no distinct genetic alterations.

**Discussion:** These results suggest that a vast majority of ER (-) cells overlying disrupted ME cell layers represent an altered clone that may be in the process of early invasion, while a few of these may be involved in a normal expansion or replenishment of the duct.

Supported by the US Army Medical Research and Materiel Command under DAMD 17-01-1-0129 and DAMD17-01-1-0130 to Yan-gao Man, MD., Ph.D.

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# **FOCAL LOSSES OF ER EXPRESSION IN EPITHELIAL CELLS AND DISRUPTIONS OF SUBJACENT MYOEPIITHELIAL CELL LAYERS ARE CORRELATED EVENTS IN ER (+) DUCTAL INTRAEPITHELIAL NEOPLASIA**

**Yan-gao Man, M.D., Ph.D., Lisa Tai, M.D., Ross Barner, M.D, Chang Y. Liang, M.D, Russell S. Vang, M.D., Jeffery S. Saenger, M.D., Kris M. Shekitka, M.D., Gary L. Bratthauer, M.S., M.T., Ping-yu Chen, and Fattaneh A. Tavassoli, M.D.**

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The purpose of this study was to assess the possible correlation between focal losses of estrogen receptor (ER) expression in epithelial (EP) cells and disruptions of subjacent myoepithelial (ME) cell layers. Consecutive sections were made from formalin-fixed, paraffin-embedded breast tissues from 220 patients with various grades of ductal intraepithelial neoplasia, and double immunostained for ER and smooth muscle actin. Cross sections of all ducts lined by  $\geq 40$  EP cells were examined for a focal ME cell layer disruption, defined as an absence of ME cells, resulting in a gap equal to or greater than the combined size of 3 EP or ME cells. A focal loss of ER expression was defined as a marked diminution or a total loss of ER staining in cells immediately overlying a disrupted ME cell layer, in contrast to strong ER expression in adjacent cells within the same duct.

Of the 220 ER (+) cases with a total of 5,698 duct cross sections examined, 94 (42.7%) contained disrupted ME cell layers with a total of 405 focal disruptions (7.1%). Of these disruptions, 350 (86.4%) were associated with a focal loss of ER expression, whereas 55 (13.6%) were subjacent to cells with a strong ER expression. The frequency of ME cell layer disruptions associated with ER (-) cells was significantly higher ( $p < 0.01$ ) than that associated with ER (+) cells. The frequency and pattern of ME cell layer disruptions were generally independent of the size, length, and architecture of the ducts. The cells overlying disrupted ME cell layers were often architecturally and morphologically indistinguishable from adjacent cells within the same duct on routine H & E stained sections.

This study suggests that a focal loss of ER expression among a group of ER (+) cells and disruption of the subjacent ME cell layer might be correlated events. As the disruption of ME cell layers are an absolute pre-requisite for tumor invasion, these events are possibly associated with progression and/or early invasion of the mammary tumors.

This study was supported by the US Army Medical Research and Materiel Command under DAMD 17-01-1-0129 and DAMD17-01-1-0130 to Yan-gao Man, MD., Ph.D.

# **FOCAL ALTERATIONS OF P27 EXPRESSION AND SUBJACENT MYOEPITHELIAL CELL LAYER DISRUPTIONS ARE CORRELATED EVENTS IN ER (-) DUCTAL INTRAEPITHELIAL NEOPLASIA**

**Yan-gao Man, M.D., Ph.D., Jeffery S. Saenger, M.D., Brian Strauss, M.D., Ph.D., Russell S. Vang, M.D., Gary L. Bratthauer, M.S., M.T., Ping-yu Chen, and Fattaneh A. Tavassoli, M. D**

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Our previous studies, using a double immunostaining technique with antibodies to smooth muscle actin (SMA) and estrogen receptor (ER), revealed that a focal loss of ER expression and disruption of a subjacent myoepithelial (ME) cell layer were correlated events in ER (+) ductal intraepithelial neoplasia (DIN). Focal disruptions of ME cell layers were also found in various grades of ER (-) DIN. This study intended to assess whether ME cell layer disruptions in ER (-) DIN may correlate with a deregulated expression of p27, a cyclin dependent kinase inhibitor that arrests cell division.

Consecutive sections were made from formalin-fixed, paraffin-embedded breast tissues from 100 patients with ER (-) DIN. Two adjacent sections were double immunostained with [1] p27 plus SMA, and [2] SMA plus a mixture of antibodies to Ki-67, Cyclin A and D3. Cross sections of all ducts lined by  $\geq 40$  EP cells were examined for focal ME cell layer disruptions and focal alterations of p27 expression, defined as a marked reduction or elevation of p27 staining in cells immediately overlying disrupted ME cell layers. The cell proliferation rates in ducts with an intact and with a disrupted ME cell layer were statistically compared.

Distinct p27 immunoreactivities were seen in a vast majority of the normal ductal and lobular cells. Although the overall level of p27 expression was generally reduced with the progression of lesions and increase of tumor histological grades, a marked reduction or total loss of p27 expression was occasionally seen in normal appearing ducts, and intense p27 immunostaining was seen in some malignant tumors. In contrast, the rate of focal alterations of p27 expression seemed to be linearly correlated with the frequency of ME cell layer disruptions in both normal appearing and neoplastic ducts. Ducts with a disrupted or no distinct ME cell layer displayed a significantly higher cell proliferation rate than ducts with an intact ME cell layer.

These findings suggest that focal alterations of p27 expression and elevated rates of ME cell layer disruptions and cell proliferation might be correlated events. Since the disruption of ME cell layer is an absolute pre-requisite for tumor invasion, elucidation of the dynamic relationship of these events and the underlying mechanism may have significant diagnostic and prognostic values.

This study was supported by the US Army Medical Research and Materiel Command under DAMD 17-01-1-0129 and DAMD17-01-1-0130 to Yan-gao Man, MD., Ph.D.

# **CO-EXPRESSION OF MASPIN AND WILMS' TUMOR 1 PROTEINS IN MAMMARY MYOEPITHELIAL CELLS--IMPLICATION FOR TUMOR PROGRESSION AND INVASION**

**Yan-gao Man, M.D., Ph.D., Russell S. Vang, M.D., Jeffery S. Saenger, M.D., Brian Strauss, M.D., Ph.D., Gary L. Bratthauer, M.S., M.T., Ping-yu Chen, and Fattaneh A. Tavassoli, M.D**

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Maspin and Wilms's tumor 1 (WT-1) proteins have been suggested as products of tumor suppressor genes, as they display inhibitory functions on tumor progression in both tissue cultures and animal models. The expression pattern and functions of these two proteins in human mammary tissues, however, have not been established. This study attempted to address these issues with an emphasis on the correlation of the proliferation rate in mammary ductal cells with the expression of these two proteins in surrounding myoepithelial (ME) cells, and with the physical integrity of ME cell layers.

Consecutive sections were made from formalin-fixed, paraffin-embedded mammary tissues from 100 patients with various grades of ductal intraepithelial neoplasia. Three adjacent sections were double immunostained with [1] smooth muscle actin plus Ki-67, [2] maspin plus Ki-67, and [3] WT-1 plus Ki-67 antibodies. The expression status of maspin and WT-1 in the same cells of each case was compared to determine the extent of co-expression of these proteins. The proliferation rates of epithelial (EP) cells in ducts with and without maspin or WT-1 expression, as well as with and without an intact ME cell layer were statistically compared.

Distinct immunostaining and the co-localization of maspin and WT-1 proteins were seen in most morphologically definable ME cells in sections from each of the 100 patients, while they were barely seen in EP or stromal cells. The expression of these proteins were closely correlated with the morphology of ME cells, but were generally independent of the size, length, or architecture of the ducts. Both morphologically normal appearing and neoplastic ducts with a reduced maspin or WT-1 expression in surrounding ME cells, or ducts with focally disrupted or no ME cell layers displayed a significantly higher cell proliferation rate than ducts with a normal maspin or WT-1 expression, and with an intact ME cell layer.

These findings suggest that maspin and WT-1 proteins may possess inhibitory functions on EP cell growth and consequently suppress progression or invasion of mammary tumors, and that maspin and WT-1 proteins might also impact the functions of ME cells. Since ME cell layers are physical barriers protecting the microenvironment and integrity of EP cells, and preventing an in situ lesion from invasion, quantitative assessments of the expression of maspin and WT-1 proteins in ME cells might have significant diagnostic and prognostic values.

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# **MORPHOLOGICALLY SIMILAR STROMAL CELLS ASSOCIATED WITH BENIGN AND MALIGNANT MAMMARY EPITHELIAL TUMORS DISPLAY DIFFERENT IMMUNOHISTOCHEMICAL AND MOLECULAR PROFILES**

**Fattaneh A. Tavassoli, M. D, Yan-gao Man, M.D., Ph.D., Brian Strauss, M.D., Ph.D., Russell S. Vang, M.D., Gary L. Bratthauer, M.S., M.T., and Ping-yu Chen.**

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Our previous studies on paraffin embedded tissues from patients with mammary and cervical carcinomas revealed high frequencies of independent and concurrent loss of heterozygosity (LOH) in microdissected epithelial (EP) tumor cells and adjacent or distant stromal (ST) cells. To confirm previous findings on a larger scale and wider spectrum, the current study attempted to compare the immunostaining pattern and the genetic profile in EP and ST cells microdissected from infiltrating syringomatous adenomas and tubular carcinomas, which are two different pathological entities, but with similar reactive background stroma.

Serial sections were made from formalin-fixed, paraffin-embedded mammary tissues from patients with above lesions, and immunostained with a panel of different antibodies. The immunostaining patterns in both the EP and ST components between two lesions were compared. Morphologically similar EP and ST cells in these lesions were microdissected for DNA extraction and assessments for LOH and microsatellite instability (MI), using PCR amplification with a panel of DNA markers at 6 different chromosomes. The frequency and pattern of LOH and MI in samples of two lesions were compared.

The cells from these two lesions displayed a substantially different immunostaining pattern to a majority of the antibodies tested, including those to tumor suppressor gene products, blood vessel components, extracellular matrix molecules, and proliferation-associated proteins. Also, both the EP and ST cells from these two lesions displayed a substantially different frequency and pattern of LOH and MI at multiple chromosomal loci, including 3p, 11p, 13p, 13q and 16q. There was no distinct LOH or MI with multiple DNA markers at chromosome 17p in the ST cells of either lesion, however.

These findings suggest that morphologically comparable ST cells associated with the benign and malignant EP lesions are bio-functionally and genetically different, but closely related with those in their EP counterparts. These findings also suggest that the functions of ST cells in both lesions are not directly subject to regulation by the p53 gene.

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# **AN ANTIGEN RETRIEVAL PROTOCOL THAT SATISFIES BOTH IMMUNOHISTOCHEMICAL AND SUBSEQUENT MOLECULAR ASSESSMENTS**

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Molecular analysis on DNA extracts from selected areas of immunohistochemically stained sections is a useful approach for studying the direct correlation between genetic and biochemical alterations. Immunohistochemical analyses of a variety of gene products in formalin-fixed, paraffin-embedded tissues, however, require a prior antigen unmasking treatment with enzymes or a high temperature using a microwave oven or a pressure cooker, which are found to substantially damage DNA and RNA structures, making subsequent genetic analyses difficult or impossible. This study attempted to develop a protocol that satisfies both immunohistochemical and genetic assessments.

Consecutive sections were made from formalin-fixed, paraffin-embedded breast tissues, and four adjacent sections were treated for antigen unmasking with [1] microwave irradiation; [2] pressure cooker incubation; [3] our modified protocol; [4] untreated. After immunostaining for a variety of cytoplasmic and nuclear antigens, comparable amounts of cells were microdissected from the same area in each of the four sections pretreated with the above four methods. Microdissected cells were subjected to DNA extraction and PCR amplification with a variety of DNA markers. Amplified PCR products among samples were semi-quantitatively compared.

Compared to other antigen unmasking methods, our protocol appeared to possess the following advantages: [1] better preservation of the morphological details; [2] a substantial reduction of the detachment of tissues from slides; [3] effectiveness on all antibodies tested; [4] consistently higher PCR yield; [5] ability to yield PCR products with higher molecular weights. The PCR efficiency in tissues treated with our protocol was comparable to those of both untreated and non-immunostained tissues. This protocol has been successfully used for the detection of over 30 different proteins that are known to require a prior antigen-unmasking treatment for their elucidation, the in situ detection of estrogen receptor mRNA, as well as both double immunohistochemical staining and subsequent molecular analyses.

This study was supported by the US Army Medical Research and Materiel Command under DAMD 17-01-1-0129 and DAMD17-01-1-0130 to Yan-gao Man, MD., Ph.D.

## **GENETIC ALTERATIONS IN ER (-) MAMMARY EPITHELIAL CELLS OVERLYING FOCALLY DISRUPTED MYOEPITHELIAL CELL LAYERS**

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To observe the dynamic alterations of myoepithelial (ME) cells in association with mammary tumor progression, a double immunostaining technique with antibodies to smooth muscle actin (SMA) and estrogen receptor (ER) was used to elucidate the ME and epithelial (EP) cells in mammary biopsies harboring ductal carcinoma in situ. Single or clusters of EP cells with a marked diminution or a total loss of ER expression were found immediately overlying focally disrupted ME cell layers, in contrast to the dominant population of ER (+) cells within the same duct that had no associated ME cell layer disruptions. This study intended to test a hypothesis that these ER (-) cells may represent a more aggressive clone that genetically differs from adjacent ER (+) cells within the same duct.

Consecutive sections were made from formalin-fixed, paraffin-embedded mammary tissues from 220 patients with various grades of ductal intraepithelial neoplasia, and double immunostained for ER and SMA. The cross sections of ducts lined by  $\geq 40$  EP cells were examined to identify ducts with focal ME cell layer disruptions. The cells overlying disrupted ME cell layers, adjacent ER (+) cells within the same duct, adjacent stromal (ST) cells, and other controls were microdissected for DNA extraction and assessment for loss of heterozygosity (LOH) and microsatellite instability (MI), using PCR amplification with 18 DNA markers at 6 chromosomes. The frequency and pattern of LOH and MI among samples were compared.

The ER (-) cells overlying disrupted ME cell layers and the adjacent ER (+) cells displayed distinct LOH and MI in each of the 18 DNA markers, with highest frequencies at chromosomes 11p and 16q. A vast majority of the cells overlying disrupted ME cell layers showed a substantially higher frequency and different pattern of LOH and MI, compared to adjacent ER (+) counterparts within the same duct. In a small proportion of cases, however, ER (-) cells showed a substantially lower frequency of LOH and MI than adjacent ER (+) cells, or even displayed no distinct genetic changes. Overall, ER (-) cells overlying disrupted ME cell layers among different foci and cases displayed a more homogeneous genetic profile than their ER (+) counterparts within the same duct.

These findings are largely in support of our hypothesis that ER (-) cells overlying disrupted ME cell layers represent a more aggressive clone, while also suggest that a few of these cells might belong to a population involving in a normal replenishment or expansion of the duct.

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monoclonal antibody for AR. Majority of the tumors, except lobular carcinoma of breast (5) and bronchoalveolar carcinoma of lung (10) were moderately to poorly differentiated. Tumors immunoreactive for > 10% of nuclei were considered AR positive. AR immunoreactivity only in the cytoplasm was interpreted as negative. Results: 56% (19/34) mammary carcinoma and 20% (2/10) adenocarcinoma of ovary were positive for AR. The other neoplasms did not show nuclear immunoreactivity for AR in > 10% nuclei; however, several of them (72%, 50/79) did show variable cytoplasmic immunoreactivity. Conclusions: The expression of AR in more than 10% nuclei in a metastatic tumor with unknown primary in a female favors mammary carcinoma; however, a remote possibility of ovarian primary cannot be ruled out.

#### 161 Relative Sustenance of Androgen Receptors Compared to Estrogen and Progesterone Receptors in Mammary Carcinoma

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**Background:** Immunoreactivity for androgen receptors (AR) is preserved more frequently than estrogen/progesterone receptors (ER/PR) in mammary carcinomas. This may be of particular value when evaluating ER/PR negative metastatic adenocarcinoma of unknown origin. The present study was undertaken to evaluate this phenomenon.

**Design:** We compared the prevalence of AR and ER/PR in mammary carcinoma. Formalin-fixed paraffin-embedded tissue sections from 34 cases of breast carcinoma [29 infiltrating ductal carcinoma (IDC), Grade III and 5 infiltrating lobular carcinoma (ILC)] were evaluated by immunohistochemistry for AR, ER, and PR with respective monoclonal antibodies. Immunoreactivity of > 10% nuclei for AR, ER, and PR respectively were considered positive. Immunoreactivity only in the cytoplasm was interpreted as negative.

**Results:** 56% of mammary carcinomas were AR positive, although 37% of these were negative for ER/PR. All ILC showed AR positivity; however, 60% of these were ER/PR negative.

	AR positive (56%, 19/34) (IDC, 48%, 14/29; ILC, 100%, 5/5)			AR negative (44%, 15/34) (IDC, 52%, 15/29; ILC, 0%, 0/5)		
	ER - Neg	PR - Neg	ER/PR both - Neg	ER - Neg	PR - Neg	ER/PR both - Neg
IDC	29%(4/14)	36%(5/14)	29%(4/14)	80%(12/15)	94%(14/15)	80%(12/15)
ILC	60%(3/5)	60%(3/5)	60%(3/5)	0%(0/0)	0%(0/0)	0%(0/0)
Total	37%(7/19)	42%(8/19)	37%(7/19)	80%(12/15)	94%(14/15)	80%(12/15)

**Conclusions:** AR expression is more frequently sustained than ER/PR in mammary carcinomas, especially in lobular variant. AR is potentially useful for the evaluation of metastatic tumor of unknown origin in women.

#### 162 Focal Loss of Estrogen Receptor (ER) Expression in ER Positive Ductal Intraepithelial Neoplasia Is Associated with Disruptions of the Immediate Subjacent Myoepithelial Cell Layer

YG Man, KM Shekita, JS Saenger, L Tai, GL Brattbauer, PY Chen, FA Tavassoli. Department of Gynecological and Breast Pathology, The Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC.

**Background:** Our previous study using double immunostaining with antibodies to ER and smooth muscle actin (SMA) revealed patchy disruptions in the myoepithelial (ME) cell layer immediately adjacent to ER negative epithelial (EP) cells in mammary ducts with ostensibly EP proliferation.

**Design:** To confirm this finding on a larger scale, the same protocol was used to assess the association between ER expression and disruptions of ME cell layers on paraffin tissue sections from 125 patients with various grades of ductal intraepithelial neoplasia. The disruption of ME cell layers is defined as widening of a ME cell layer gap equal to the diameter of at least 3 EP cells in the cross section of a given duct. Focal loss of ER expression is defined as a significant reduction or complete loss of ER expression in a cluster of EP cells immediately overlying the disrupted ME cell layer, compared to strong ER expression in the remaining neoplastic cells within the same duct. The total number of the cross sections of ducts with proliferative changes was counted. All profiles with disrupted ME cell layers were photographed, and prints were made at a magnification of 400-800X for immunohistochemical and morphological assessments.

**Results:** Of the 125 cases, 62 (49.6%) showed disrupted ME cell layers; 246 (6.6%) disruptions were detected from 3,733 evaluated duct cross sections. Of the 62 cases with disrupted ME cell layers, 40 (64.5%) contained less than 4 and 22 (35.5%) displayed more than 4 disruptions. Of these disruptions, 225 (91.5%) from 59 cases were associated with focal loss of ER expression and 21 (8.5%) from 9 cases were subject to ER positive cells. The frequency and pattern of disruptions was generally independent of the size of ducts or the degree of neoplasia. The cells overlying the ME disruptions were generally morphologically indistinguishable from adjacent neoplastic cells within the same duct on routine H&E sections.

**Conclusions:** These findings suggest that focal loss of ER expression might play an important role in tumor progression and that double immunostaining with SMA and ER could assist in detection of incipient cancer invasion.

**Acknowledgement:** This study is supported by Congressionally Directed Medical

Research Programs, The Idea Award (BC001187) and Career Development Award (BC001186) to Yan-gao Man, MD., Ph.D..

#### 163 Atypical Ductal Hyperplasia in Juvenile Hypertrophy of the Breast

S Mangray, JF Simpson, RA Jensen, DL Page. Vanderbilt University Medical Center, Nashville, TN.

**Background:** Atypical ductal hyperplasia (ADH) is unusual in young women and development of carcinoma has not been seen with limited follow up (Eliassen et al., Am J Surg Pathol 1992;16(3): 246-51). We sought to determine the incidence of ADH in juvenile hypertrophy encountered in a large breast pathology consultation service.

**Design:** The files of the Breast Consultation Service at Vanderbilt University Medical Center were searched for patients aged 16 to 25 years who were diagnosed with ADH, using strict previously published criteria, for the period 1996 to August 2001. The surgical pathology reports and corresponding hematoxylin and eosin slides were reviewed. Histologically confirmed cases of juvenile hypertrophy were selected and follow up data was reviewed.

**Results:** A total of 377 cases from women in this age group were received in consultation during the study period. Nineteen cases (5.0%) of ADH were diagnosed. Five of the nineteen cases were in women with juvenile hypertrophy undergoing bilateral mastoplasty, two of whom had bilateral disease and three of whom had unilateral disease. In most cases the pattern of ADH was largely lobulocentric, however in 2 cases there was limited extension of ADH into an adjacent duct. Follow-up data ranging from 2-63 months showed no development of carcinoma.

**Conclusions:** Much longer follow-up is required to determine whether patients with ADH in the setting of juvenile hypertrophy are at increased risk for the development of breast carcinoma. Furthermore, caution is needed in order not to over-diagnose these cases as carcinoma in situ. A conservative approach to management, with close follow up, is indicated.

#### 164 Pagetoid Spread in Ductal Carcinoma In Situ: Characterization and Computer Simulation

KD Mannes, ME Edgerton, JF Simpson, RA Jensen, DL Page. Vanderbilt University Medical Center, Nashville, TN.

**Background:** Pagetoid proliferation of neoplastic cells in ducts adjacent to foci of ductal carcinoma in situ is frequently observed in breast biopsies. However, its risk implications are unclear. If this histologic finding actually represents the spread of ductal carcinoma in situ within ducts, then pagetoid proliferation might be useful as a marker of greater extent of ductal carcinoma in situ. Pagetoid proliferation may also be useful as an indicator of the functional characteristics of tumor cells that impact clinical events.

**Design:** Seventy cases designated as pagetoid spread of neoplastic cells in association with ductal carcinoma in situ were obtained from the Vanderbilt Breast Consult Service and the lesions and associated findings were characterized. A cell automata model simulating the spread of ductal carcinoma in situ was utilized to study diffusion and proliferation as independent parameters in pagetoid extension.

**Results:** In approximately 60% of cases, the ductal carcinoma in situ was more than 1 cm in greatest extent or was described as "extensive" and in less than 10% of cases was smaller than 5 mm. The vast majority of cases in which margin status was assessed had inadequate margins of resection with 44% having transected margins and 50% having a margin of less than 5 mm. All patterns of ductal carcinoma in situ were represented, although comedo subtype was uncommon. Computer simulations showed pagetoid spread becomes extensive when diffusion rates are multiple orders of magnitude greater than proliferation rates.

**Conclusions:** Ductal carcinoma in situ associated with pagetoid proliferation of neoplastic cells along the ducts was often extensive with positive or close surgical margins in excisional biopsies. The computer simulation of pagetoid spread within ducts suggests that the diffusion to proliferation ratio must be high in order for ductal carcinoma in situ to extend in this fashion.

#### 165 Lobular Carcinoma In Situ on Core Needle Biopsy: When Should One Recommend Excision?

LP Middleton, S Grant, T Stephens, N Sneige, AA Sahin. The University of Texas M.D. Anderson Cancer Center, Houston, TX.

**Background:** Core needle biopsy (CNB) is the preferred technique for evaluating breast masses and abnormal mammographic findings. The frequency of detection of noninvasive lobular lesions by CNB is increasing. Historically, the diagnosis of lobular carcinoma in situ (LCIS) has been considered a risk factor for the development of invasive carcinoma, and treatment has consisted of careful follow-up with or without tamoxifen. The purpose of this study was to review CNB material with the primary diagnosis of LCIS, atypical lobular hyperplasia (ALH), and lobular neoplasia (LN) in conjunction with clinical and radiological findings to make recommendations as to when excision is merited.

**Design:** The M.D. Anderson pathology database was searched from 1995 to 2001 for CNB cases with LCIS, ALH, and LN as the primary diagnosis. Microcalcifications



**Primary bilateral breast cancers display different LOH and CGH profiles in both epithelial and stromal components.**

Man YG, Moirfar F, Shekitka KM, Stamatakis M, Lininger RA, Kuhls E, Brattbauer GL, Tavassoli FA. Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC.

One of our previous studies revealed that morphologically similar cells from two sides of bilateral primary breast cancers displayed a different pattern and frequency of loss of heterozygosity (LOH), suggesting that these might be independent lesions. This study attempted to confirm previous findings. **Methods:** The frequency and pattern of LOH and DNA copy numbers in microdissected epithelial (EP) and stromal (ST) cells from left and right lesions of 20 synchronous and metachronous breast cancers were compared, using PCR and comparative genomic hybridization (CGH) techniques. **Results:** A total of 147 LOH were detected in a total of 122 paired informative foci with a combination of 9 markers. Of 147 LOH, 82 (56%) were seen in the left and 65 (44%) in the right lesions. Of 122 paired foci, 97 (80%) showed unilateral and 25 (20%) displayed bilateral LOH. Of 42 paired, microdissected samples, 32 (76%) displayed more independent, while 4 (10%) showed more concurrent LOH. In 7 selected cases, CGH changes (gains or losses) were detected in the EP component in one side of three cases and in both sides of three cases, and loss of 11q was seen in the ST component in one side of two cases. CGH changes were distributed in 5 left and 4 right lesions, but none of cases that showed CGH changes displayed an identical pattern or frequency of changes in either EP or ST component of both breasts. **Conclusions:** These results are in favor of independent lesions in most primary bilateral cancers, and further suggest that ST cells are concurrently involved or even play initiative roles in development and progression of some breast cancers.

## **PUBLICATIONS**

**Allelic Losses at 3p and 11p are Detected in Both Epithelial and Stromal Components of Cervical Small-Cell Neuroendocrine Carcinoma.** Yan-gao Man, M.D., Ph.D., Ciaran Mannion, M.D., Elizabeth Kuhls, M.D., Farid Moinfar, M.D., Gary L. Bratthauer, M.S., M.T., Jorge Albores-Saavedra, M.D., and Fattaneh A. Tavassoli, M.D. *Applied Immunohistochemistry & Molecular Morphology*

**Multiple Use of Slab Gels in Sequencing Apparatus for Separation of Polymearase Chain Reaction Products.** Yan-gao Man, M.D., Ph.D., Elizabeth Kuhls, M.D., Gary L. Bratthauer, M.S., M.T., Farid Moinfar, M.D., and Fattaneh A. Tavassoli, M.D. *Electrophoresis*

**An Improved Method for DNA Extraction for Paraffin Sections.** Yan-gao Man, M.D., Ph.D., Elizabeth Kuhls, M.D., Gary L. Bratthauer, M.S., M.T., Farid Moinfar, M.D., and Fattaneh A. Tavassoli, M.D. *Pathology Research and Practice*

**Contributions of Intercalated Duct Cells to the Normal Parenchyma of Submandibular Glands of Adult Rats.** Yan-gao Man, M.D., Ph.D., William D. Ball, Luigi Marchetti, and Arthur Hand. *The Anatomical Record*

**Combined E-Cadherin and High Molecular Weight Cytokeratin Immunoprofile Differentiates Lobular, Ductal, and Hybrid Mammary Intraepithelial Neoplasias.** Gary L. Bratthauer, M.S., M.T., Farid Moinfar, M.D., Michael D. Stamatakis, LT COL, USAF, MC, Thomas P. Mezzetti, LCDR, MC, USNR, Kris M. Shekitka, COL, USAF, MC, Yan-gao Man, M.D., Ph.D., and Fattaneh A. Tavassoli, M.D. *Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology*

**Androgen and Estrogen Receptor mRNA Status in Apocrine Carcinomas.** Gary L. Bratthauer, M.S., M.T., Rugh A Lininger, M.D., Yan-gao Man, M.D., Ph.D., and Fattaneh A. Tavassoli, M.D. *Diagnostic Molecular Pathology*

**Morphofunctional Features of Intraductal Hyperplasia, Atypical Intraductal Hyperplasia, and Various Grades of Intraductal Carcinoma.** Yan-gao Man, M.D., Ph.D., and Fattaneh A. Tavassoli, M.D. *The Breast Journal*

## Assessing the value of p16 in the diagnosis of malignant melanoma

Ailing Li, MD, MS<sup>1</sup>, Marjorie Fowler, MD<sup>1</sup>, Yan-Gao Man MS, MD, PhD<sup>2</sup>,

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2. Armed Forces Institute of Pathology.

**Background:** The p16 (CDKN2A/MTS-1) gene, a member of the tumor suppressor gene family, encodes a protein that regulates the checkpoint of cell cycles from the G1 to the S phase. A mutation or deletion at the p16 locus has been found in about 36% of malignant melanomas. The diagnostic value of p16 expression in melanoma cells has not been investigated extensively.

**Design:** The expressions of p16 detected by immunohistochemical staining with the use of monoclonal antibody (p16<sup>ink4a</sup> Ab-4, labvision.com) were compared in three types of cutaneous melanocytic tumors which were common melanocytic nevi (20 cases), atypical melanocytic nevi (16 cases) and malignant melanomas (15 cases). A cell proliferation rate marker, Ki67, was also evaluated in the study as a control marker (>10% as positive). Chi-square tests were used for statistical analysis.

**Results:** Positive p16 staining was found in 95% of common melanocytic nevi (19/20) and in 75% of atypical melanocytic nevi (12/16). However, no melanoma in-situ (0/4) and only 18% of melanomas were p16 positive (2/11). The difference in p16 expression among common melanocytic nevi, atypical melanocytic nevi and malignant melanomas was statistically significant ( $\chi^2=26.1$ ,  $p<0.000001$ ). Positive Ki67 staining was not found in common melanocytic nevi (0/20) or in atypical melanocytic nevi (0/16), but was seen in 40% of malignant melanomas (6/15). The difference in Ki67 expression among common melanocytic nevi, atypical melanocytic nevi and malignant melanomas was statistically significant ( $\chi^2=16.8$ ,  $p=0.0003$ ). It is interesting to note that when evaluating the samples with the two markers in combination all p16 positive specimens (33) were Ki67 negative, and 33% of p16 negative samples (6/18) were Ki67 positive.

**Conclusion:** p16 has a high specificity for distinguishing common melanocytic nevi (95%), a relatively high specificity for atypical melanocytic nevi (75%) and a relatively high sensitivity for diagnosing malignant melanomas (87%). Ki67 and p16 are inversely correlated. However, combining the two markers does not improve the sensitivity or specificity.

**Discussion:** The study suggests that p16 might be a useful molecular marker for aiding in the diagnosis of malignant melanoma. However, this is a small pilot study and this finding needs to be confirmed by larger studies.

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五言 慰友人

1979年春於湖北武漢东湖月

悉一知己因遭女友抛弃而伤悉，随作此诗以慰。

採花有何难？何处无花园！

牡丹虽稀疏，野菊遍山川。

合欢夜隐去，桃李晨争艳。

百花纵凋残，吐蕊有来年。

耕播如蓝山<sup>①</sup>，自有花似锦。

垂钓渭水畔<sup>②</sup>，携来美女还！

①传说中佛祖如来之居所。

②传太公姜子牙曾於渭水河畔以无饵直钩於距水面三尺处垂钓，并高吟：“负命者上钩来”。结果钓来周文王之女姬子周武王及周朝数百年江山。

卜算子 西山怀古

1977年秋於湖北鄂城西山寺

湖北鄂城县西北有一高丘，名西山，山中有一古寺。相传顶盛时寺中僧人逾百。宋代词人苏东坡任职黄州时常到此与众僧人讲好友指点江山，激扬文字。然笔者造访时，此处已面目全非，一片凄凉。

寺门枯藤遮，

神案蛛网裹。

试问东坡何处去，

寺中黑鸦舞。

灶台青苔铺，

饮池早干枯。

后院香笋突起处，

顶出人头骨。

## 英汉直译引起的三则笑话

“Excite”在英汉词典中的主要释义为：兴奋、刺激，是日常生活用语中使用频率最高的单词之一。然而，十五年前我首次使用此词时却闹出了个至今仍令我羞得耳根发红的笑话。

此时，我以访问学者身份在 Georgetown 大学微生物系从事科研工作。此年圣诞夜，导师 Rosenthal 教授夫妇邀我并妻到其家共进晚餐。导师富有且慷慨，备有中西佳肴二十余盘及名酒二十余种。导师家人并二十余位男女亲朋均善饮。我奉陪4~5杯后即觉不胜酒力，随即坐在身旁的师母索桔汁代酒。师母好客，一再劝我再喝几杯。我因酒后易烦躁不安，极难入睡而推辞：“No, I can not. If I drink too much, I will be very excited and not be able to sleep”。导师及师母对我的托辞十分好奇，两人不约而同地问道：“Really?”。我小之答道：“Yes! My wife can verify that”。未待二人发问，我妻子已主动答道：“Yes! He is always very excited whenever he has a heavy drink, and will stay awake all night long”。我们的交谈顷刻就吸引了房中所有人的注意力。凡对男女用词为惊奇的眼光上下打量我与妻子，更多的人则对我们挤眉弄眼，作怪象。导师则起身离座站在我身后，双手搭在我肩上，用一种阴阳怪气的语调对我说：“Dr. Man, do not worry, your wife is here”。他的话音未落，众人已哄堂大笑。约半数宾客甚至笑得前仰后合，捂着肚子跑到客厅。

饭后，我私下问导师众人哄笑之因。导师解释：Excite一词有多重含义。在饮酒时用此词则意含说话人怒火攻心，斗生‘趣冲冲。导师的解释使我很快领悟到我与妻子席间言论的确切英文含意竟是：我每次喝多酒后总是斗生怒暴增，与妻缠绵通宵达旦。

二、突受惊吓后，人们常会有心跳或心悸感。若事出人为，受害者则会责怪肇事者：“你把我吓出心脏病了，我的心都快被吓碎了”。对于国人这一表达方式，美国人似有截然不同的理解。下引本人十二年前在 Howard 大学医学院工作时一亲身经历为证。

一日我在实验室阅读组织切片，突闻脑后一声巨响。由于实验室位于地下层，一向极为宁静，加之注意力高度集中，这突如其来的响声惊得我不由自主地从座椅上一跃而起，心中怦怦直跳。回头一望，先是室中的女技术员把一个巨大的空玻璃杯摔碎在地。此女世代居美，祖、父辈均为政府高级职员，其本人持一各牌大学学士文凭，聪明伶俐，但做事极马虎，粗心，曾数次因不循实验程序而惹我不快。此事自令我光火，便冲着她没好气地说：“Being careful! You have almost broken my heart”。她以狐疑的眼光望着我，几次张口却未发出声音。我气以与她各道道歉，但一时未能找出合适的措词，便随口说道：“Forget it”。岂料她似甚委屈地嘟囔道：“I did not break your heart, maybe your girl friend did”。一听此言，我本已基本平息的火气又陡然窜起。我伸出左手，朝她吼道：“Yes, you did. Check my pulse, my heart beats at least 130 times a minute”。她望了望我那恼怒的面神，伸了下舌头，做了个鬼脸便是开了。

十余分钟后，她主动找到我，很有礼貌地说：“Mr. Man, I am very sorry for what I did. But, you should have said that ‘you have scared my to death’ rather than ‘you have almost broken my heart’”。我问书何，她解释道：前句话意指你受到惊吓后而感到不快。而后句话则表示你对我钟情万分，爱慕有加，而我却根本不爱及你。书此，你深感失意、悲伤。听完她解释，我顿觉脸上发烫，无地自容。她看出了这一点，忙说：“Mr. Man, you are a very nice and handsome young man. I do love you”。我虽她是在逢场作戏，但她那温柔的话语，甜蜜的笑容已将我心中的不快一扫而净。

三. 在日常生活用语中, 国人常用衣服 (clothing) 一词泛指各类服装, 如衬衫、外套、裤子等。美国人对此却分得很细。若忽略此差异, 则可能闹出笑话。

十二年前, 美国朋友 Kurt Nauss 先生邀我到他家做客。因此时天寒地冻, 我穿了一件厚厚的大衣。推开他家门后, 只见他与太太正忙于准备饭菜。我不忍让他放下手中的活计来为我挂衣, 便问道: "Kurt, may I take my clothing off and put in your closet?" Nauss 先生闻言忙跑过来, 低声对我说: "Sorry, you can not". 我反问道: 室内温度比室外高得多, 何不能脱衣? 他很有礼貌地答道: "No matter how hot it is, it is a very bad idea to take off your clothing in front of people". 我们之间虽熟识, 且曾在他家相聚数次, 但望着他那严肃认真的神情, 我只好入境随俗, 穿着大衣走向客厅。Nauss 先生叫住我: "Mr. Man, although you are not allowed to take your clothing off, you are welcome to take your coat off". 我忙答道: 这正是我刚才所求的。他笑着说: 你刚才根本没提外套 (coat), 你所说的照我理解是, 你想脱光衣服, 一条不挂。